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Transcatheter aortic valve implantation reduces plasma concentrations of TMAO and indoxyl sulfate: a prospective, multicenter cohort study

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Abstract

Background: *Intestinal microbial metabolites, such as trimethylamine-N-oxide (TMAO) and indoxyl sulfate (IS), have been suggested as markers for the progression of aortic stenosis (AS). However, the impact of transcatheter aortic valve implantation (TAVI) on these intestinal bacterial metabolites has not been evaluated in a multicenter clinical study. The aim of this study was to determine the effect of TAVI on plasma levels of intestinal bacterial metabolites and to assess the predictive value of these metabolites for major adverse cardiovascular events (MACE) following TAVI.*

Methods: *Consecutive patients with AS referred for TAVI were enrolled in this study. Blood samples were collected one day before TAVI and at hospital discharge. The concentrations of intestinal microbial metabolites were measured using ultra performance liquid chromatograph coupled with a mass spectrometer.*

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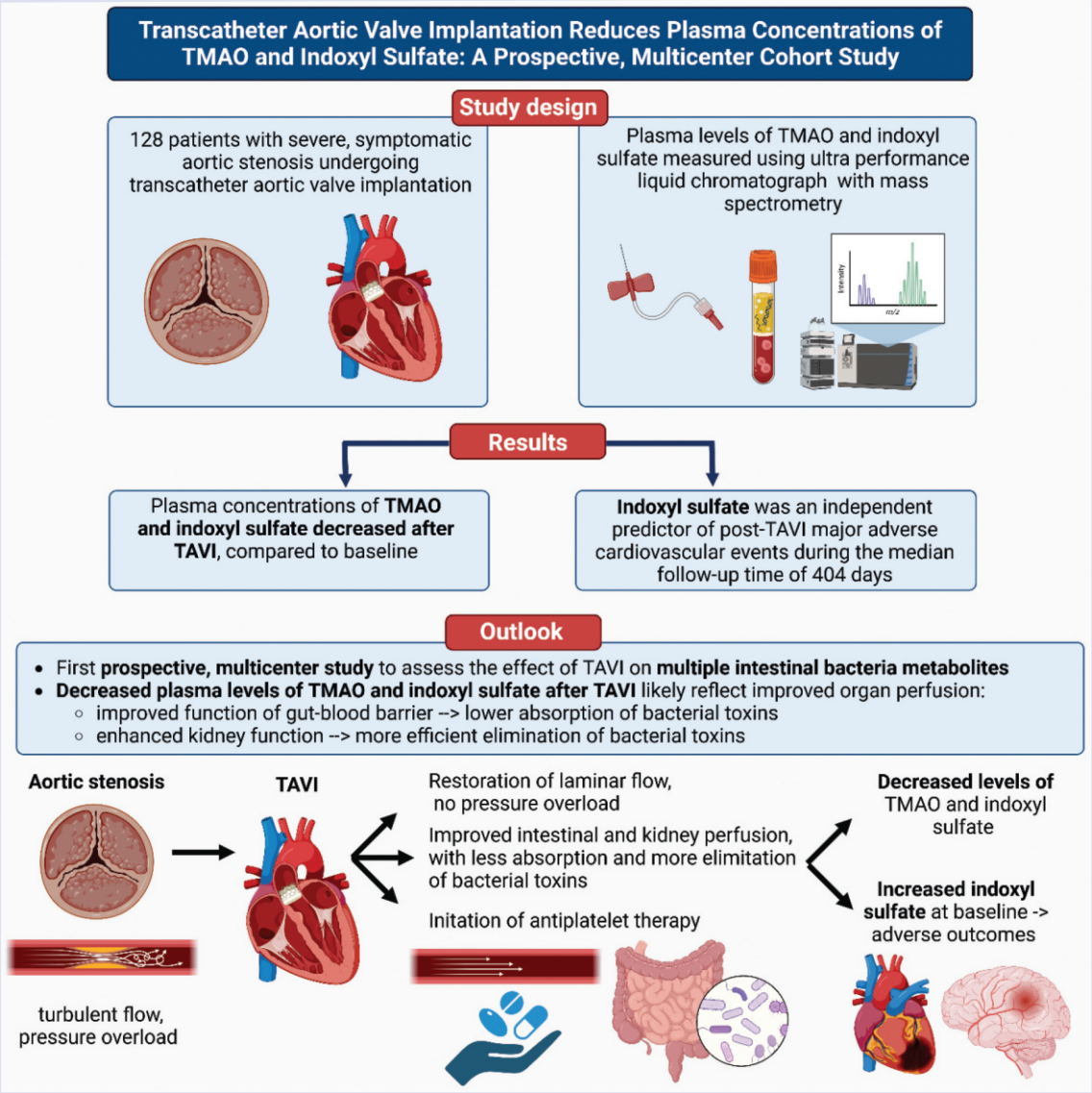
Results: *Plasma levels of TMAO and IS decreased after TAVI, compared to baseline ($p \leq 0.004$ for all). Among 128 patients included in the study, 21 patients (16.4%) developed MACE during the median follow-up time of 404 days. Baseline plasma IS level was higher in patients with MACE, compared to those without MACE ($p = 0.001$). Increased baseline IS level predicted MACE with 75.0% sensitivity and 74.3% specificity independent of other clinical variables (OR 14.264, 95% CI 3.442–59.117, $p < 0.001$) and decreased the chance of event-free survival ($p_{log\ rank} < 0.001$).*

Conclusions: *Plasma concentrations of TMAO and IS decreased after TAVI, compared to baseline. Elevated plasma IS levels were associated with a 14-fold increase in the odds of post-TAVI MACE during a median follow-up period of 404 days.*

Keywords: aortic stenosis, intestinal metabolites, indoxyl sulfate, transcatheter aortic valve implantation, trimethylamine-N-oxide

Central figure. Summary of the study results



TAVI — transcatheter aortic valve implantation; TMAO — trimethylamine-N-oxide

Introduction

Aortic stenosis (AS) is the most commonly acquired valvular heart disease in the Western world and is a significant healthcare challenge [1], with an increasing prevalence along with aging populations [2]. There are two methods of treating severe, symptomatic AS: surgical aortic valve replacement (SAVR) and transcatheter aortic valve implantation (TAVI). The choice of method is based on the patient age and the risk of surgical mortality. TAVI is recommended in older patients (> 75 years of age), who are at a high risk of surgical mortality (Society of Thoracic Surgeons Predicted Risk of Mortality [STS-PROM] or EuroSCORE II > 8%) or otherwise unsuitable for surgery [3]. In the remaining patients, the choice between SAVR and TAVI should be made by a Heart Team based on clinical and anatomical evaluation, weighing the risks and benefits of each approach for an individual patient [3]. Despite excellent outcomes after TAVI with 85% of patients free from structural valve deterioration at eight years [4], about 25% of patients experience major and minor major adverse cardiovascular and cerebrovascular events (MACE) after TAVI. Currently, there are no clinically applicable biomarkers allowing the prediction of such events.

Numerous studies have shown an association between the plasma concentrations of toxins produced by intestinal microbiota and cardiovascular diseases (CVD) [5–9]. Among these toxins, indoles and trimethylamines have been the focus of extensive research in CVD [5, 10].To reach systemic circulation, bacterial toxins must cross the gut-blood barrier, the permeability of which has been shown to increase in CVD [6, 8, 10]. Most toxins, such as trimethylamine and indole, are metabolized by the liver into less toxic or no-toxic compounds, such as TMAO and indoxyl sulfate, respectively, and are subsequently removed by the kidneys [6, 9, 11].

Although there is growing evidence suggesting the contribution of primary microbiota-produced toxins such as TMA and indole, it is their final metabolites, such as TMAO and IS, that are mostly studied in clinical research. These metabolites are more stable and easier to measure, making them more reliable markers. Thus, the plasma concentrations of TMAO and IS serve as proxies for their toxic precursors' production, the function of the gut-blood barrier, liver metabolism, and kidney function.

Previously, it was shown that plasma concentration of TMAO is an independent predictor of adverse outcomes in patients after acute myocardial infarction [12]. Recently, it was demonstrated that patients with severe AS had higher TMAO levels compared to patients without AS, which was associated with increased two-year all-cause mortality after TAVI [13]. However, the effect of TAVI on intestinal bacteria metabolites and their predictive value for post-TAVI outcomes have not hitherto been evaluated in a multicenter clinical study. As TAVI restores physiological hemodynamic conditions, it is associated with improved organ perfusion, including that of the gut, as well as anti-inflammatory and antithrombotic effects [14, 15]. The present study hypothesized that TAVI decreases plasma concentrations of microbiota-derived metabolites and that the plasma levels of these metabolites are associated with clinical outcomes. The goal of this study was

1. to determine the effect of TAVI on plasma level of intestinal bacteria metabolites, and
2. to evaluate the predictive value of these metabolites for MACE after TAVI.

Methods

This is a prospective, multicenter study conducted at three academic centers in Poland (Warsaw, Poznan, Nowa Sol) in collaboration with the Department of Experimental Physiology and Pathophysiology, Medical University of Warsaw, , Poland. The study protocol was approved by the Ethics Committee of Medical University of Warsaw (approval number: KB/128/2018, KB/4/A2021).

Patients diagnosed with severe AS and qualified for TAVI based on the Heart Team's decision were recruited. Severe AS was defined as aortic valve area (AVA) < 1.0 cm^2 or indexed AVA < 0.6 cm^2/m^2, calculated by the continuity equation on transthoracic echocardiography (TTE). In patients with low-flow, low-gradient AS and reduced LVEF, dobutamine stress echocardiography was performed to differentiate between true severe AS and pseudo-severe AS, and in patients with low-flow, low-gradient AS and preserved LVEF, computed tomography was performed to assess aortic valve calcium score [3]. Exclusion criteria were transcatheter valve-in-valve implantation, chronic kidney disease (estimated glomerular filtration rate [eGFR] < 30 mL/min), autoimmune diseases, active neoplastic disease, pregnancy, breast-feeding.

TAVI was performed by an interventional cardiologist (J.K., Z.H., B.R.) and a cardiothoracic surgeon (R.W.) in a hybrid operating room. According to

the 2017 ESC guidelines on valvular heart disease, applicable when conducting this study for three-six months, followed by single antiplatelet therapy (mostly ASA) in patients with no indication for oral anticoagulation (OAC), or OAC if required [16]. Other drugs were continued at the discretion of the treating physician, according to individual comorbidities.

Blood samples were collected to ethylenediaminetetraacetic acid (EDTA) plastic tubes (S-Monovette, Sarstedt) at two time points: one day before TAVI and at hospital discharge after TAVI. Platelet-depleted plasma was prepared by double centrifugation (2500 g, 15 min, 20°C, acceleration speed one, no brake). The supernatant was transferred into Eppendorf tubes and stored in −80°C until analyzed. Plasma concentrations of TMAO and IS were measured using a Waters Acquity Ultra Performance Liquid Chromatograph coupled with a Waters TQ-S Triple-Quadrupole Mass Spectrometer. The mass spectrometer operated in the multiple-reaction monitoring (MRM)-positive electrospray ionization (ESI) mode, as previously described [12].

Patient data were collected during the index hospitalization for TAVI. A follow-up visit in the outpatient clinic was scheduled after one year ± three months, when control TTE was performed and data regarding MACE (all-cause death, cardiovascular death, myocardial infarction, stroke, transient ischemic attack [TIA], decompensation of heart failure, need for re-intervention) were recorded.

The primary end-point was the change in plasma concentration of TMAO from baseline to post-TAVI. The secondary end-points were the changes in plasma concentrations of IS from baseline to post-TAVI and the predictive value of TMAO and IS for the occurrence of MACE during the follow-up.

The sample size for the primary endpoint was calculated for the mean difference in primary endpoint, based on the previous study, where patients with severe AS had two-fold higher TMAO levels compared to patients without AS [17]. The required sample size was calculated by a two-sided t-test at a significance level of 0.05, assuming the mean difference between the groups = 1, SD in each group ± 2.0, and nominal test power = 0.9. Based on this sample size estimation, a total of 86 patients should be enrolled in the study to observe a mean difference in TMAO concentration from baseline to post-TAVI. Taking into account that up to 30% of patients can be potentially lost to follow-up, at least 120 patients should be included in the study.

Statistical analysis was conducted using IBM SPSS Statistics, version 27.0 (IBM, New York, USA). Categorical variables were presented as number and percent and compared using Chi-square test. The Shapiro-Wilk test was used to assess normal distribution of continuous variables. Continuous variables were presented as mean with standard deviation (SD) or median with interquartile range (IQR). Changes in metabolite concentration before and after TAVI were calculated with Wilcoxon signed-rank. To assess the difference in variables between patients with and without MACE, the Student t-test and U-Mann Whitney test were used to compare data with and without normal distribution, respectively. The Spearman correlation coefficient was used to evaluate correlations between plasma levels of intestinal bacteria metabolites and echocardiographic parameters of AS severity. The predictive value of intestinal bacteria metabolites for MACCE and the cut-offs were calculated using a receiver operating characteristic (ROC) curve. The Logistic regression model incorporating intestinal bacteria metabolites concentration and clinical characteristics which predicted MACE at $p < 0.1$ in the univariable analysis were used to determine independent predictors of MACE. The results of univariable and multivariable regression analyses are reported as odds ratio (OR) and 95% confidence interval (CI). A two-sided p-value below 0.05 was considered significant.

Results

Between November 2018 and September 2021, 128 patients were enrolled in the study with complete follow-up regarding mortality, based on the National Insurance Database, and 8 patients lost to follow-up regarding other adverse events. The median (IQR) time between baseline blood collection and TAVI was one day and between TAVI and second blood collection was five days. The median time to follow-up was 404 (194–510 days). Twenty-one patients (16.4%) developed MACE during the follow-up: four all-cause deaths, five cardiovascular deaths, one TIA, eight decompensation of heart failure, one need for reintervention six months after the first TAVI due to severe paravalvular leakage (PVL) and two valve thromboses (one symptomatic and one subclinical, with the latter diagnosed due to increased transvalvular gradients on follow-up echocardiography and confirmed in angiographic computed tomography). Among eight patients with decompensated heart failure, seven patients had heart failure with preserved

ejection fraction (HFpEF), five had mild PVL and four moderate mitral regurgitation. None of these patients had severe PVL or severe aortic or mitral regurgitation. Comparison of baseline characteristics between patients who experienced post-TAVI MACE and those with did not is shown in Table 1. Except for older age ($p < 0.001$) in patients who developed MACE, there were no differences regarding comorbidities, laboratory, echocardiographic, procedural parameters, periprocedural complications and pharmacotherapy at discharge between the groups.

Plasma concentrations of TMAO and IS decreased after TAVI, compared to baseline ($p \leq 0.004$ for all; Fig. 1A, 1B). In the subgroup of patients who experienced MACE, plasma concentrations of TMAO were comparable before and after TAVI ($p = 0.233$), whereas plasma concentrations of IS decreased after TAVI, compared to baseline ($p = 0.003$). There was a weak, negative correlation between the baseline plasma concentrations of TMAO and mean aortic valve gradient ($r = –0.206$, $p = 0.027$). There were no significant correlations between plasma concentrations of IS and AS severity parameters (Tab. 2).

Baseline plasma concentration of TMAO was comparable in patients with and without MACE, whereas plasma concentration of IS was higher in patients who experienced MACE during the follow-up period, compared to patients without MACE ($p = 0.001$; Fig. 2A–D).

Increased pre-TAVI plasma IS concentration above the cut-off value, defined as 1650 ng/dL plasma based on the ROC curve, predicted MACE with 75.0% sensitivity and 74.3% specificity (AUC 0.73, $p = 0.001$). In univariable analysis, baseline IS concentration > 1650 ng/dL, age, mean aortic gradient and treatment with renin-angiotensin-aldosterone system (RAAS) were predictive of MACE (Suppl. Tab. 1). In multivariable Cox regression analysis, only IS concentration > 1650 ng/dL and age were independent predictors of MACE (OR 14.264, 95% CI 3.442–59.117, $p < 0.001$ for IS; OR 1.183, 95% CI 1.027–1.363, $p = 0.020$ for age, respectively; AUC 0.83, $p < 0.001$; Tab. 3, Suppl. Fig. 1).

Kaplan-Meier analysis demonstrated that patients with baseline plasma concentration of IS > 1650 mg/dL had a lower chance of event-free survival during the follow-up, compared to patients with concentrations of IS below the cut-off concentration ($p < 0.001$ for the log-rank test; Fig. 3). The study results are summarized in the Central Figure.

Discussion

This study is the first to examine the effect of TAVI on plasma concentrations of TMAO and IS, which are derived from intestinal microbiota metabolism, and to evaluate the predictive value of these compounds for post-TAVI MACE. The main finding of the current study is that plasma concentration of TMAO decreased after TAVI compared to baseline. Furthermore, it was found that plasma concentrations of IS decreased after TAVI as well, and that IS is an independent predictor of post-TAVI MACE during the median follow-up time of 404 days.

Previous studies demonstrated that plasma TMAO concentration is increased both in patients with atherosclerotic CVD and AS, compared to healthy controls, which can be explained by the involvement of an active "atherosclerosis-like" process in the initiation phase of degenerative AS [13, 18]. However, the effect of TAVI on plasma levels of TMAO has not been previously studied. The present study showed that plasma concentrations of TMAO, but also IS decreased in AS patients after TAVI, compared to baseline levels. There are several potential explanations for this finding, including improved function of the gut-blood barrier and/or enhanced kidney function responsible for the elimination of these compounds due to improved organ perfusion after TAVI. Additionally, the initiation of antiplatelet therapy after TAVI cannot be excluded as a contributing factor.

According to the hypothesis of the heart-bowel axis, AS is associated with intestinal ischemia, which leads to impaired intestinal function, including a leaky gut [19, 20]. Restoration of physiological hemodynamic conditions by TAVI improves intestinal blood supply and function, which may be reflected by the decreased plasma levels of TMAO and IS observed in our study [19]. Whether SAVR exerts the same impact on intestinal bacterial metabolites, has not been hitherto investigated. Currently being conducted is a prospective, observational TASTE (TMAO in severe Aortic STenosis: association with Echocardiographic, biochemical and histopathological indices of heart failure) study, with the goal to evaluate the relationship between baseline TMAO concentrations and changes in clinical status, echocardiographic and biochemical parameters after interventional treatment of severe AS (either TAVI or SAVR) [21].

In contrast to the previous study [17], the present study did not demonstrate that TMAO is an independent predictor of adverse outcomes in

Table 1. Comparison of baseline characteristics between patients who experienced major adverse cardiovascular events and those with did not during the follow-up period

	Total (n = 128)	No MACE (n = 107)	MACE (n = 21)	p-value
Baseline characteristics (median, IQR or n, %)				
Age, years	79.5 (74.0–83.0)	79.0 (74.0–82.0)	83.0 (81.0–86.0)	0.001
Gender, male	57 (44.5)	44 (41.1)	13 (61.9)	0.108
BMI, kg/m^2	26.1 (24.5–30.0)	26.2 (24.5–30.1)	26.0 (24.5–28.5)	0.578
Euroscore II, %	3.7 (2.5–5.1)	3.8 (2.4–5.1)	3.7 (3.1–5.1)	0.991
Co-morbidities (n, %)				
Hypertension	103 (83.1)	85 (82.5)	18 (85.7)	0.722
Diabetes mellitus	48 (38.7)	42 (40.8)	6 (28.6)	0.281
Atral fibrillation	42 (33.9)	35 (34.0)	7 (33.3)	0.954
Prior stroke/TIA	12 (9.7)	9 (8.7)	3 (14.3)	0.433
Prior myocardial infarction	32 (25.8)	26 (25.2)	6 (28.6)	0.751
Prior PCI	56 (45.2)	45 (43.7)	11 (52.4)	0.466
Prior CABG	9 (7.3)	8 (7.8)	1 (4.8)	0.629
COPD	14 (11.3)	10 (9.7)	4 (19.1)	0.218
Heart failure (NYHA ≥ III)	60 (46.9)	52 (48.6)	8 (38.1)	0.378
CKD > 3a	25 (20.2)	19 (18.5)	6 (28.6)	0.292
Laboratory data (median, IQR or mean, SD)				
Haemoglobin, g/dL	12.2 (4.1)	12.1 (4.5)	12.7 (2.5)	0.900
Creatinine, mg/dL	55.0(43.0–70.0)	55.5(44.0–70.0)	51.0(43.0–62.0)	0.320
NT-proBNP, pg/mL	1993	1908	2266	0.705
	(566.0–3718)	(559–3901)	(684–3707)	
eGFR, mL/min	55 (43–70)	56 (44–70)	51 (43–62)	0.320
Echocardiography before TAVI (median, IQR)				
Ejection fraction, %	55 (45–60)	55 (46–60)	60 (45–66)	0.091
V max, m/s	4.2 (3.8–4.5)	4.2 (3.8–4.4)	4.3 (4.1–4.5)	0.251
Gradient max, mmHg	72.0 (61.9-82.0)	71.0 (64.0–83.0)	72.0 (46.0–82.0)	0.690
Gradient mean, mmHg	42.7 (33.0-51.0)	43.0 (34.4–52.0)	41.5 (26.0–46.0)	0.158
AVA (VTI) , cm^2	0.8 (0.6–0.9)	0.8 (0.6–0.9)	0.7 (0.6–0.9)	0.550
AVAi, cm^2/m^2	0.4 (0.4–0.5)	0.4 (0.4–0.5)	0.4 (0.4–0.5)	0.930
Procedural characteristics (n, %)				
Femoral access	118 (95.9)	99 (97.1)	19 (90.5)	0.338
Valve type				0.105
EvolutR	42 (34.2)	38 (37.3)	4 (19.1)	
EvolutPRO	10 (8.1)	9 (8.8)	1 (4.8)	
Portico	33 (26.8)	22 (21.6)	11 (52.4)	
Accurate Neo	11 (8.9)	9 (8.8)	2 (9.5)	
Accurate Neo 2	24 (19.5)	21 (20.6)	3 (14.3)	
Hydra	2 (1.6)	2 (2.0)	0 (0.0)	
Navitor	1 (0.8)	1 (1.0)	0 (0.0)	
In-hospital complications (n, %)				
Life-threatening or disabling bleeding*	11 (8.6)	9 (8.4)	2 (9.5)	0.868
Major vascular complication*	8 (6.3)	7 (6.5)	1 (4.8)	0.758
New pacemaker	10 (7.8)	8 (7.4)	2 (9.5)	0.797
In-hospital mortality	2 (1.6)	0 (0.0)	2 (9.5)	0.028

Echocardiography at follow-up (median, IQR)				
Ejection fraction, %	60 (4–60)	59 (50–60)	60 (40–65)	0.588
V max, m/s	2.0 (1.7–2.3)	2.0 (1.6–2.3)	2.1 (1.9–2.4)	0.140
Gradient max, mmHg	17.0 (12.0–21.0)	17.0 (12.0–21.0)	19.0 (15.0–24.0)	0.095
Gradient mean, mmHg	8.0 (6.0–10.0)	8.0 (6.0–10.0)	9.0 (7.0–12.0)	0.156
AVA (VTI) , cm^2	1.9 (1.7–2.1)	1.9 (1.7–2.2)	1.9 (1.6–2.0)	0.550
AVAi, cm^2/m^2	1.0 (0.9–1.2)	1.0 (0.9–1.2)	1.1 (0.9–1.2)	0.930
Paravalvular aortic regurgitation (n, %)				
Mild	59 (46.1%)	49 (45.8%)	10 (47.6%)	0.878
Moderate	9 (7.0%)	9 (8.4%)	0 (0.0%)	0.354
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Post-TAVI pharmacotherapy (n, %)				
Acetylsalicylic acid	81 (68.1)	71 (70.3)	10 (55.6)	0.217
P2Y12 inhibitor	86 (72.9)	74 (74)	12 (66.7)	0.519
Oral anticoagulant	53 (44.9)	43 (43)	10 (55.6)	0.324
Beta-blockers	98 (83.8)	83 (83.8)	15 (83.3)	0.957
RAAS inhibitor	76 (63.9)	61 (60.4)	15 (83.3)	0.062
MRA	31 (27)	29 (29.6)	2 (11.8)	0.126
Loop diuretics	102 (85.7)	85 (84.2)	17 (94.4)	0.251
Statins	101 (85.6)	85 (85)	16 (88.9)	0.665

AVA — aortic valve area; AVAi — aortic valve area index; BMI — body mass index; CABG — coronary artery bypass graft surgery; COPD — chronic obstructive pulmonary disease; CKD — chronic kidney disease; eGFR — estimated glomerular filtration rate; CRP — C-reactive protein; MACE — major adverse cardiovascular events; MRA — mineralocorticoid receptor antagonists; NT-proBNP — N-terminal pro B natriuretic peptide; NYHA — New York Heart Association; PCI — percutaneous coronary intervention; RAAS — renin-angiotensin-aldosterone system; TAVI — transcatheter aortic valve implantation; TIA — transient ischemic attack

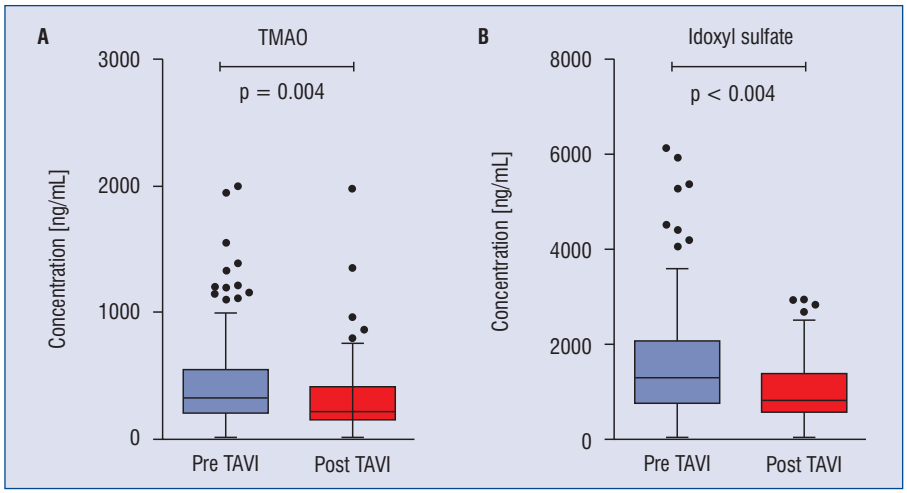


Figure 1. Plasma concentrations of intestinal bacteria metabolites before and after transcatheter aortic valve implantation. TAVI — transcatheter aortic valve implantation; TMAO — trimethylamine-N-oxide

Table 2. Correlations between baseline plasma concentrations of intestinal bacteria metabolites and echocardiographic parameters of aortic stenosis severity. Significant correlations are made in bold and marked with a star

Metabolite (ng/dL)	Gradient max (mmHg)	Gradient mean (mmHg)	AVAi (cm^2/m^2)
TMAO	r = –0.169	r = –0.206*	r = 0.051
IS	r = –0.150	r = –0.176	r = 0.010

AVAi — aortic valve area index; IS — indoxyl sulfate; TMAO — trimethylamine-N-oxide; * p < 0.05, **p < 0.01, ***p < 0.001

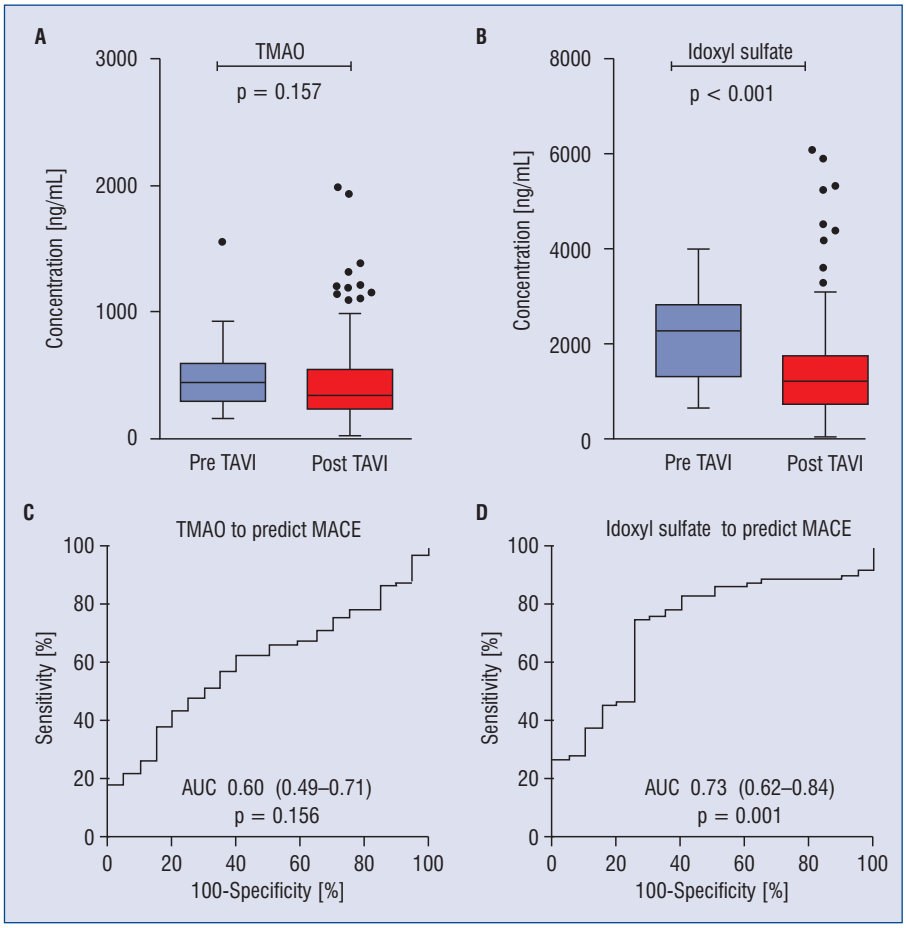


Figure 2. Plasma concentrations of intestinal bacteria metabolites measured before transcatheter aortic valve implantation (TAVI) in patients who did and who did not experience major adverse cardiovascular events (MACE) during the follow-up period (panel A, B) and receiver operating characteristic curves (ROC) to predict MACE after TAVI (panel C, D). TMAO — trimethylamine-N-oxide

Table 3. Results of multivariable analysis to predict major adverse cardiovascular events after transcatheter aortic valve implantation using the concentration of indoxyl sulfate above the cut-off value and clinical variables

	OR	95% CI		p-value
		Lower	Upper	
Indoxyl sulfate, >1650 ng/dL	14.264	3.442	59.117	< 0.001
Age, years	1.183	1.027	1.363	0.020
Gradient mean, mmHg	1.109	0.931	1.320	0.247
RAAS inhibitor	4.392	0.926	20.832	0.062

CI — confidence interval; OR — odds ratio; RAAS — renin-angiotensin-aldosterone system

AS patients undergoing TAVI. These discrepancies might be due to various factors, including demographic differences, diet, and pharmacotherapy, which affect plasma TMAO levels [13, 22], potentially confounding the results. Since neither the current study nor the previous studies collected data on all these factors, the reasons for the inconsistent results remain to be defined. Hence, further studies are needed.

Another reason of decreased TMAO levels after TAVI might be due to the initiation of dual antiplatelet therapy with ASA and clopidogrel in the majority

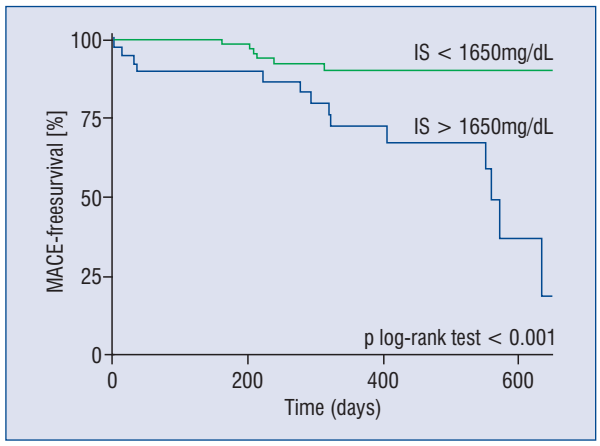


Figure 3. Kaplan-Meier survival analysis for major adverse cardiovascular events (MACE) after transcatheter aortic valve implantation in patients with baseline plasma concentration of indoxyl sulfate (IS) above and below the cut-off value of 1650 ng/dL, determined on the receiver operating characteristic curve

of these patients, according to the previous ESC guidelines on valvular heart disease, was applicable when conducting this study. Even low-dose ASA was shown to partly reduce choline supplement-dependent rise in plasma TMAO, probably by altering intestinal microbiota composition [23], which might have also contributed to altered TMAO levels measured in these patients 5–6 days after TAVI.

IS has emerged as a potent cardiotoxin and uremic toxin and a strong predictor of cardiovascular mortality in patients with chronic kidney disease (CKD) [11, 24]. AS and CKD share many risk factors. In a large-scale observational cohort study including 1,121,875 Stockholm citizens without a prior diagnosis of AS, moderate and severe CKD (eGFR < 45 mL/min) was associated with 20% higher risk of AS during a median follow-up of 5.1 years [25]. IS might provide the pathophysiological link between CKD and AS, being involved in the osteogenic differentiation of human valvular interstitial cells from the aortic valve and subsequent valve calcification [26, 27]. IS also has pro-fibrotic, pro-hypertrophic and pro-inflammatory effects on cardiac fibroblasts and myocytes [28], as well as prothrombotic effects by inducing platelet hyperactivity [29]. Plasma IS level predicts cardiovascular events in patients with chronic heart failure, dilated cardiomyopathy and acute coronary syndrome, independent of CKD [24, 30, 31]. The results of the study herein revealed that elevated IS concentration along with age was the only independent predictor of adverse outcomes in AS patients undergoing TAVI, predicting MACE with 75.0% sensitivity and 74.3% specificity. The link between elevated IS concentration and post-TAVI MACE requires further investigation. One hypothesis could be that the proinflammatory and prothrombotic effects of IS contribute to adverse outcomes, such as cardiovascular deaths, TIA or valve thrombosis, observed in the present study. However, this study is too small to analyze this relationship in sufficient detail, leaving the pathophysiological role of elevated IS level in patients with AS undergoing TAVI unclear.

Among other clinical characteristics, only age was an independent predictor of adverse outcomes. The lack of a relationship between the classic CV risk factors and MACE does not seem associated with drop-outs or in-hospital complications, since 120 patients had complete follow-up regarding MACE and the rate of procedural complications were comparable between the groups. However, the baseline characteristics suggested that the present cohort was relatively low-risk (median EuroScore II 3.7%, median LVEF 55%), despite their advanced age (median 79.5 years). Hence, the lack of association between classic CV risk factors and adverse outcomes might potentially be explained by the good clinical status pre-TAVI.

The current study has several limitations. First, plasma levels of TMAO and IS were measured only twice — before and after TAVI, which does not allow drawing any conclusions regarding the long-term changes in the concentrations of these metabolites during the follow-up period, and neither with their association to MACE. Hence, the intestinal bacterial metabolites might have been affected by confounders such as periprocedural antibiotic prophylaxis, length of fasting, altered in-hospital diet, intensive care stay, the stress of the procedure and hospitalization. Therefore, AS repair should not be considered a sole reason for the change in intestinal bacterial metabolite dynamics in patients hospitalized for TAVI. Second, the number of MACE was relatively low, and the confidence interval for the predictive value of IS for MACE is broad, which requires caution when interpreting the results. Third, no data was collectedregarding acute kidney injury, infections or other problems which might have affected the long-term outcomes. Fourth, all TAVI procedures were done by experienced high-volume teams, which eliminated the bias due to various expertise levels, but might limit the general results' applicability.

Conclusions

Plasma concentration of TMAO and IS decreased after TAVI, compared to baseline, likely reflecting the restoration of physiological hemodynamic conditions. Increased baseline plasma IS concentrations were associated with 14-fold higher odds of MACE during the median follow-up time of 404 days, as previously reported for other cardiovascular diseases. Considering numerous other peri-procedural factors affecting intestinal bacterial metabolite levels in patients undergoing TAVI, the results should be interpreted with caution and require confirmation in future studies.

Data availability statement: Source data are available upon request to the corresponding author.

Ethics statement: All procedures were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients included in the study.

Author contribution: All authors contributed to this study. Conceptualization, design and methodology: A.G., M.U.; Investigation and resources: A.G., M.Grabowski, B.Rymuza., Z.H., J.K., R.W., M.K., J.B., J.H., A.O.-W., M.Grygier, K.J.F., M.U. Data Curation: A.G., M.Gniot, B.Rajewska., W.D., W.K., E.B., J.M.Z., M.U.; Writing — original draft preparation: A.G., M.Gniot, B.Rajewska, W.D., W.K., E.B., J.M.Z.; Writing — review and editing: A.G., M.Gniot., B.Rajewska, W.D., W.K., E.B., J.M.Z., M.Grabowski, B.Rymuza, Z.H., J.K., R.W., M.K., J.B., J.H., A.O.-W., M.Grygier, K.J.F., M.U.

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Supplementary material: Supplementary Table 1, Supplementary Figure 1.

References

1. Iung B, Vahanian A. Epidemiology of valvular heart disease in the adult. Nat Rev Cardiol. 2011; 8(3): 162–172, doi: 10.1038/nrcardio.2010.202, indexed in Pubmed: 21263455.
2. Bonow RO, Greenland P. Population-wide trends in aortic stenosis incidence and outcomes. Circulation. 2015; 131(11): 969–971, doi: 10.1161/CIRCULATIONAHA.115.014846, indexed in Pubmed: 25691712.
3. Vahanian A, Beyersdorf F, Praz F, et al. ESC/EACTS Scientific Document Group, ESC/EACTS Scientific Document Group, ESC/EACTS Scientific Document Group, ESC/EACTS Scientific Document Group, ESC/EACTS Scientific Document Group. 2021 ESC/EACTS Guidelines for the management of valvular heart disease. Eur J Cardiothorac Surg. 2021; 60(4): 727–800, doi: 10.1093/ejcts/ezab389, indexed in Pubmed: 34453161.
4. Shimamura J, Takemoto S, Fukuhara S, et al. Long-term outcomes after transcatheter aortic valve replacement: Meta-analysis of Kaplan-Meier-derived data. Catheter Cardiovasc Interv. 2023; 102(7): 1291–1300, doi: 10.1002/ccd.30854, indexed in Pubmed: 37890015.
5. Gebrayel P, Nicco C, Al Khodor S, et al. Microbiota medicine: towards clinical revolution. J Transl Med. 2022; 20(1): 111, doi: 10.1186/s12967-022-03296-9, indexed in Pubmed: 35255932.
6. Nowiński A, Ufnal M. Gut bacteria-derived molecules as mediators and markers in cardiovascular diseases. The role of the gut-blood barrier. Kardiol Pol. 2018; 76(2): 320–327, doi: 10.5603/KP.a2017.0204, indexed in Pubmed: 29131297.
7. Tomasova L, Grman M, Ondrias K, et al. The impact of gut microbiota metabolites on cellular bioenergetics and cardiometabolic health. Nutr Metab (Lond). 2021; 18(1): 72, doi: 10.1186/s12986-021-00598-5, indexed in Pubmed: 34266472.
8. Nowiński A, Ufnal M. Trimethylamine N-oxide: A harmful, protective or diagnostic marker in lifestyle diseases? Nutrition. 2018; 46: 7–12, doi: 10.1016/j.nut.2017.08.001, indexed in Pubmed: 29290360.
9. Huć T, Nowinski A, Drapala A, et al. Indole and indoxyl sulfate, gut bacteria metabolites of tryptophan, change arterial blood pressure via peripheral and central mechanisms in rats. Pharmacol Res. 2018; 130: 172–179, doi: 10.1016/j.phrs.2017.12.025, indexed in Pubmed: 29287686.
10. Ufnal M, Zadlo A, Ostaszewski R. TMAO: A small molecule of great expectations. Nutrition. 2015; 31(11-12): 1317–1323, doi: 10.1016/j.nut.2015.05.006, indexed in Pubmed: 26283574.
11. Hung SC, Kuo KL, Wu CC, et al. Indoxyl Sulfate: A Novel Cardiovascular Risk Factor in Chronic Kidney Disease. J Am Heart Assoc. 2017; 6(2), doi: 10.1161/JAHA.116.005022, indexed in Pubmed: 28174171.
12. Gąsecka A, Fidali O, Kłębukowska A, et al. Plasma concentration of TMAO is an independent predictor of adverse outcomes in patients after acute myocardial infarction. Postepy Kardiol Interwencyjnej. 2023; 19(1): 31–39, doi: 10.5114/aic.2022.123884, indexed in Pubmed: 37090218.
13. Kocyigit D, Tokgozoglu L, Gurses KM, et al. Association of dietary and gut microbiota-related metabolites with calcific aortic stenosis. Acta Cardiol. 2021; 76(5): 544–552, doi: 10.1080/00015385.2020.1853968, indexed in Pubmed: 33334254.

14. Baratchi S, Zaldivia MTK, Wallert M, et al. Transcatheter Aortic Valve Implantation Represents an Anti-Inflammatory Therapy Via Reduction of Shear Stress-Induced, Piezo-1-Mediated Monocyte Activation. Circulation. 2020; 142(11): 1092–1105, doi: 10.1161/CIRCULATIONAHA.120.045536, indexed in Pubmed: 32697107.
15. Wilimski R, Budzianowski J, Łomiak M, et al. Extracellular Vesicles to Predict Outcomes After Transcatheter Aortic Valve Implantation - a Prospective, Multicenter Cohort Study. J Cardiovasc Transl Res. 2024; 17(5): 992–1003, doi: 10.1007/s12265-024-10521-x, indexed in Pubmed: 38807003.
16. Baumgartner H, Falk V, Bax JJ, et al. ESC Scientific Document Group, ESC Scientific Document Group. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. Eur Heart J. 2017; 38(36): 2739–2791, doi: 10.1093/eurheartj/ehx391, indexed in Pubmed: 28886619.
17. Guo Y, Xu S, Zhan H, et al. Trimethylamine N-Oxide Levels Are Associated with Severe Aortic Stenosis and Predict Long-Term Adverse Outcome. J Clin Med. 2023; 12(2), doi: 10.3390/jcm12020407, indexed in Pubmed: 36675336.
18. Carità P, Coppola G, Novo G, et al. Aortic stenosis: insights on pathogenesis and clinical implications. J Geriatr Cardiol. 2016; 13(6): 489–498, doi: 10.11909/j.issn.1671-5411.2016.06.001, indexed in Pubmed: 27582763.
19. Fülöp P, Dvorožňáková M, Vachalcová M, et al. [Gut microbiome in heart failure and aortic stenosis]. Vnitr Lek. 2022; 68(E-2): 4–10, indexed in Pubmed: 36208939.
20. Janeiro MH, Ramírez MJ, Milagro FI, et al. Implication of Trimethylamine N-Oxide (TMAO) in Disease: Potential Biomarker or New Therapeutic Target. Nutrients. 2018; 10(10), doi: 10.3390/nu10101398, indexed in Pubmed: 30275434.
21. Gąsecka A, Rzepa Ł, Konwerski M, et al. Trimethylamine-N-oxide (TMAO) versus echocardiographic, biochemical and histopathological indices of heart failure in patients with severe aortic stenosis: Rationale and design of the prospective, observational TASTE study. Cardiol J. 2022; 29(4): 691–697, doi: 10.5603/CJ.a2022.0023, indexed in Pubmed: 35470417.
22. Thomas MS, Fernandez ML. Trimethylamine N-Oxide (TMAO), Diet and Cardiovascular Disease. Curr Atheroscler Rep. 2021; 23(4): 12, doi: 10.1007/s11883-021-00910-x, indexed in Pubmed: 33594574.
23. Zhu W, Wang Z, Tang WH, et al. Gut Microbe-Generated Trimethylamine -Oxide From Dietary Choline Is Prothrombotic in Subjects. Circulation. 2017; 135(17): 1671–1673, doi: 10.1161/CIRCULATIONAHA.116.025338, indexed in Pubmed: 28438808.
24. Imazu M, Fukuda H, Kanzaki H, et al. Plasma indoxyl sulfate levels predict cardiovascular events in patients with mild chronic heart failure. Sci Rep. 2020; 10(1): 16528, doi: 10.1038/s41598-020-73633-9, indexed in Pubmed: 33020564.
25. Vavilis G, Bäck M, Occhino G, et al. Kidney Dysfunction and the Risk of Developing Aortic Stenosis. J Am Coll Cardiol. 2019; 73(3): 305–314, doi: 10.1016/j.jacc.2018.10.068, indexed in Pubmed: 30678761.
26. Candellier A, Issa N, Grissi M, et al. Stop-As Investigators. Indoxyl-sulfate activation of the AhR- NF- B pathway promotes interleukin-6 secretion and the subsequent osteogenic differentiation of human valvular interstitial cells from the aortic valve. J Mol Cell Cardiol. 2023; 179: 18–29, doi: 10.1016/j.yjmcc.2023.03.011, indexed in Pubmed: 36967106.
27. Delgado-Marin M, Sánchez-Esteban S, Cook-Calvete A, et al. Indoxyl Sulfate-Induced Valve Endothelial Cell Endothelial-to-Mesenchymal Transition and Calcification in an Integrin-Linked Kinase-Dependent Manner. Cells. 2024; 13(6), doi: 10.3390/cells13060481, indexed in Pubmed: 38534325.
28. Lekawanvijit S, Adrahtas A, Kelly DJ, et al. Does indoxyl sulfate, a uraemic toxin, have direct effects on cardiac fibroblasts and myocytes? Eur Heart J. 2010; 31(14): 1771–1779, doi: 10.1093/eurheartj/ehp574, indexed in Pubmed: 20047993.
29. Yang Ke, Du C, Wang X, et al. Indoxyl sulfate induces platelet hyperactivity and contributes to chronic kidney disease-associated thrombosis in mice. Blood. 2017; 129(19): 2667–2679, doi: 10.1182/blood-2016-10-744060, indexed in Pubmed: 28264799.
30. Shimazu S, Hirashiki A, Okumura T, et al. Association between indoxyl sulfate and cardiac dysfunction and prognosis in patients with dilated cardiomyopathy. Circ J. 2013; 77(2): 390–396, doi: 10.1253/circj.cj-12-0715, indexed in Pubmed: 23100090.
31. Watanabe I, Tatebe J, Fujii T, et al. Prognostic Utility of Indoxyl Sulfate for Patients with Acute Coronary Syndrome. J Atheroscler Thromb. 2019; 26(1): 64–71, doi: 10.5551/jat.44149, indexed in Pubmed: 29780075.